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Effects of Chemical Seed Treatments of Soybean Bacterial Pustule, on Rhizobium Symbiosis and Yield of Soybean

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ABSTRACT

Seven chemical compounds, including antibiotics and fungicides, namely Aureomycin, Dumocycline, Agrimycin-100, Terramycin, Tersan 75, Thane M-45 and Cupravit, used in seed treatment of soybean to control bacterial pustule, were examined for their effects on Rhizobium nodule bacteria both under laboratory and field trials, at Kasetsart University Bangkhen Campus and Suwan Farm respectively. In the laboratory, chemicals applied at 250 ppm inhibited the growth of 5 strains of *Rhizobium japonicum,* namely USDA 8-0, USDA 15-7, USDA 110, USDA 122 and TH7. An exception was found with Agrimycin-100 which inhibited the growth of Rhizobium strain TH7, only above 1,500 ppm. In a field test, the same 7 chemicals were used as a seed treatment of soybean varieties SJ2, SJ4 and SJ5, both with and without inoculation with Rhizobium. The results revealed no significant differences in fresh nodule weight and 100-seed weight. Seeds treated with fungicides had significantly higher number of plants per unit area and seed yield compared with seeds treated with antibiotics or untreated seeds. Among soybean varieties, only inoculated seeds of SJ4 had higher yield than without Rhizobium inoculation.

INTRODUCTION

The use of chemical seed treatments has facilitated the control of many soybean diseases elsewhere (Adair *et al,* 1950 ; Nemlienko and Kulik, 1960 ; Krasnova, 1963 ; Muras, 1964 ; Thapliyal and Misra, 1974) but there has been little use of seed treatment in Thailand. Because of the seriousness of soybean bacterial pustule in Thailand, experiments were initiated to test the effectiveness of various methods of chemical application, including foliar spray (Prathuangwong and Choakhen, 1984) and seed dressing (Prathuangwong and Choochoa, 1988), for control of this disease. Foliar application of fungicides to control bacterial pustule on soybean plant had been successful while seed treatment with chemicals produced questionable results, possibly because Thai famers are using seeds inoculated with Rhizobium. The objectives of this research were to determine the effectiveness of chemical seed treatments on the seed borne incidence of soybean bacterial pustule and the effect on the Rhizobium symbiosis. The effect of chemicals on soybean bacterial pustule had been reported earlier (Prathuangwong and Choochoa, 1988) and the effect of these chemicals on Rhizobium strains, nodulation and plant growth is presented in this paper.

MATERIALS AND METHODS

Seeds were collected from three soybean varieties (SJ 2, SJ 4, and SJ 5) which had been

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previously inoculated with bacterial pustule pathogen *(Xanthomonas campestris* pv. *glycines)*. A laboratory experiment was undertaken at Bangkhen Campus, Kasetsart University, Bangkok and field trials at the National Maize and Sorghum Research Center (Suwan Farm), Pakchong, Nakhon Ratchasima province.

Laboratory experiment

Rhizobium strains : Rhizobium nodule bacteria, *Rhizobium japonicum,* were multiplied from direct transfer of pure culture and from organic matter mixed with culture obtained from the Bacteriology and Soil Microbiology Section, Plant Pathological Research Division, Depertment of Agriculture, Ministry of Agriculture and Cooperatives. Five Rhizobium strains (USDA 8-0, USDA 15-7, USDA 110, USDA 122 and THA 7) were maintained on Yeast Manital Agar (YMA : 0.5 g K_2HPO_4 ; 0.2 g $MgSO_4.7H_2O$; 0.1 g Nacl ; 0.5 g yeast extract ; 10.0 g manital ; 15 g agar ; 1000 ml distilled water ; adjusted pH to 6.5 - 6.8) for both pure culture and mixed organic matter culture and kept in a refrigerator.

Chemical treatments ; The seven chemicals used to treat bacterial pustule infestation of soybean seeds included 4 antibiotics ; Aureomycin (chlorotetracycline HCl), Dumocycline (tetracyline HCl), Agrimycin – 100 (oxytetracycline) and 3 fungicides ; Thane M-45 (manganese 16%, zine 2% ethylene bisdithiocarbamate ion, manganese ethylene bisdithio-carbamate plus zine ion 1.2%), Tersan 75 (tetramethylthiuram disulfide 75%) and Cupravit [$Cu(OH_2)$, $CuCl_2$ oxychloride 86-88%]. The effect of these chemicals on bacterial pustule was reported earlier (Prathuangwong and Choochoa, 1988). Initial experiments were conducted to study the relative ability of these same chemicals to impede the growth of Rhizobium strains in the laboratory.

A stock solution at 2500 ppm was prepared for each chemical. Two ml of the mixture was pipetted and placed in a flask containing 18 ml melted YMA. When the temperature decreased to 45-50°C, the chemical and agar medium was thoroughly blended ; the mixture was then poured into sterilized petridishes. Each dish, therefore, contained agar medium and the chemical at a concentration of 250 ppm.

Each Rhizobium strain was cultured on YMA slant in the test tube for 7 days until white slimy colonies covered the whole media surface. Then 5 ml of sterilized distilled water was pipetted into the Rhizobium culture tube. A sterilized loop was employed to scrape Rhizobium colonies from the agar surface. The test tube was then shaken in the vibrator to distribute the pathogen into the solution. After that, 0.1 ml of the solution was pipetted and dropped in the test tube containing 10 ml of sterilized distilled water and was shaken in the vibrator until thoroughly mixed. Then 0.2 ml of the mixture was again pipetted into the petridish containing 250 ppm of the prepared chemical. A " L " glass rod was use to smear the pathogen evenly over the nutrient surface. When the medium was dry, the dish was turned upside down and kept at room temperature for 7-10 days before the colonies were counted.

Any chemical which exerted no effect on Rhizobium growth at 250 ppm, was further tested at concentrations of 500, 750, 1000, 1500, 2000 and 2500 ppm by the same procedures.

Field experiment

Seeds of soybean varieties, namely SJ2, SJ4 and SJ5, which were infected with bacterial pustule and had been treated with the 7 chemicals, were retreated with Rhizobium bacteria contained in a dust powder, obtained from the Bacteriology and Soil Microbiology Section, Division of Plant Pathology, Department of Agriculture at the rate of 200 gm/5 kg seed weight (Vasuwat, 1979). Rhizobium used in the field experiment were the same strains used in the laboratory which had already tested with chemicals. Four control treatments prepared for each soybean variety. Control-1 was treated with Rhizobium and adjuvant (Shellestral), control-2 was treated with sterilized distilled water and Rhizobium, while control-3 and control-4 were treated with adjuvant (Shellestral) and sterilized distilled water only, respectively. The treated seeds were then planted in the field at Suwan Farm. An RCB design with 4 rows in a 2 × 4 m sub-plot, with 50 × 25 cm spacing and 4 seeds/hole for each variety, with 3 replicates, was used in the experiment. The number of seedlings in the two middle rows of each subplot, except the first and the last holes, was counted 3 weeks after planting.

Fresh Rhizobium nodules were measured at 45 days after planting on soybean roots which were randomly dug from every forth hole in the 2 outer rows. Only one plant was taken from each hole, making a total of 10 plants per subpot.

Soybean seed was harvested from the two middle rows, except the first and the last plant of each subplot. After the seeds were shelled and cleaned, 100 gms were randomly selected from each subplot for measuring the moisture content by a hygrometer. The 4 duplicate samples of 100 seeds were sampled from every subplot to calculate the average weight of 100 seeds at 13% moisture content (m.c.) and also seed yields for each subplot were calculated at 13% m.c.

The experiment was conducted for two consecutive growing seasons, the rainy (Aug - Nov, 1984) and the dry (Jan - Apr, 1985) seasons, using the same procedures.

RESULTS

Effects of chemical seed treatments on the growth of Rhizobium strains in the laboratory

It was found that the 7 chemicals tested, including control 1 (Shellestral), inhibited the growth of every strain of Rhizobium, except for Agrimycin-100, which did not reduce the growth of strain THA 7 at a concentration of 250 ppm.

The testing of higher concentrations of Agrimycin against Rhizobium strain TH 7 revealed that at 1000 ppm Rhizobium growth of individual colonies was reduced eventhough the number of colonies did not decrease. The development of each colony at 1000 ppm was slower than at a concentration of 500 ppm, while at 1500 ppm each colony grew more slowly than at 1000 ppm. When the concentration increased to 2000 and 2500 ppm, the growth of colonies were completely inhibited.

Effects of chemical seed treatments on Rhizobium nodule bacteria and crop growth in the field

(1) Plant establishment

The different varieties of soybean responded differently to chemical treatments. For soybean variety SJ2 the highest number of plants established in the field during the rainy season (Aug-Nov, 1984), was obtained from seed applications of 1000 ppm and 2000 ppm of Tersan 75 and 500 ppm of Aureomycin (Table 1). Significantly lower numbers of plants were established, compared to the control treatments. With SJ4, the application of 1000 and 2000 ppm of Thane M-45 and 1000 and 2000 ppm of Tersan 75 gave significantly ($p < 0.05$) higher plant establishment than untreated control treatments. The highest plant densities were achieved from the lower concentration of Tersan 75 (191, 677 plants/ha) and the higher concentration of Thane M-45 (192,500 plants/ha). Significantly ($P < 0.05$) lower plant densities, compared to the control treatments, occurred from the application of 1000 ppm of Dumocycline (76,666 plants/ha). With SJ5, the application of 1000 and 2000 ppm of Thane M-45 and 1000 and 2000 ppm of Tersan 75 gave significantly ($P < .05$) higher plant establishment than untreated control treatments. Terramycin at 500 ppm and Agrimycin-100 at 1000 ppm also gave significantly higher plant densities. Tersan 75 at 1000 ppm gave the highest plant densities (214,167 plants/ha). Dumocycline at 1000 ppm gave the lowest plant density in this variety (62,500 plants/ha) but this was not significantly lower than control treatment.

Table 1 Number of plants/hectare of 3 soybean varieties after seed treatment with chemicals and Rhizobium for controlling bacterial pustule during rainy season.

Chemicals	Varieties		
	SJ2	SJ4	SJ5
	No. of Plants/hectare		
Aureomycin 500 ppm	148,333 a	139,167 d	120,833 fghi
Aureomycin 1,000 ppm	84,166 efg	133,333 d	96,666 ij
Dumocycline 500 ppm	100,833 def	106,667 e	137,500 efg
Dumocycline 1,000 ppm	60,833 g	76,666 f	62,500 k
Terramycin 500 ppm	111,667 bcde	167,500 c	164,167 bcd
Terramycin 1,000 ppm	105,000 cdef	102,500 e	105,833 hi
Agrimycin-100 500 ppm	110,000 bcde	162,500 c	112,500 ghi
Agrimycin-100 1,000 ppm	108,333 bcdef	172,500 bc	175,833 bc
Tersan 75 1,000 ppm	154,167 a	191,667 a	214,167 a
Tersan 75 2,000 ppm	149,167 a	165,000 c	175,833 bc
Thane M-45 1,000 ppm	112,500 bcde	185,000 ab	188,333 b
Thane M-45 2,000 ppm	144,167 ab	192,500 a	158,333 cde
Cupravit 1,000 ppm	130,000 abcd	166,667 c	135,833 efg
Cupravit 2,000 ppm	140,833 abc	165,000 c	140,833 def
Control 1	84,166 efg	103,333 e	130,000 fgh
Control 2	72,500 fg	102,500 e	78,333 jk
Control 3	106,667 cdef	122,500 fghi	124,167 fghi
Control 4	95,833 ef	145,833 ab	110,000 bcde
CV (%)	16.92	5.34	10.53

Means in each column followed by the same letters are not significantly different (P = 0.05) according to DMRT.

During the dry season there was no significant ($P < .05$) increase in plant establishment from chemical seed treatments applied to SJ2 and SJ4, compared to the best control treatments, but with the SJ5 variety, Tersan 75 applied at 1000 ppm gave significantly ($P < .05$) higher plant density (210,000 plants/ha). Tersan 75 at 1000 ppm also gave the highest plant densities for the other varieties. No chemical seed treatments significantly decreased plant density, compared to the worst control treatment, but Dumocycline at 1000 ppm gave consistently the lowest plant densities for all varieties.

Table 2 **Number of plants/hectare of 3 soybean varieties after treatment with chemicals and Rhizobium for controlling bacterial pustule during dry season.**

Chemicals	Varieties		
	SJ2	SJ4	SJ5
	No. of Plants/hectare		
Aureomycin 500 ppm	152,500 a	142,500 abcde	129,167 cdef
Aureomycin 1,000 ppm	88,333 bcd	139,167 abcde	103,333 efg
Dumocycline 500 ppm	104,167 abcd	115,833 cde	146,667 bcde
Dumocycline 1,000 ppm	64,166 d	84,166 e	70,833 g
Terramycin 500 ppm	115,833 abcd	178,333 abcd	173,333 abc
Terramycin 1,000 ppm	108,333 abcd	114,167 de	116,667 defg
Agrimycin-100 500 ppm	115,000 abcd	170,833 abcd	121,667 defg
Agrimycin-100 1,000 ppm	111,667 abcd	184,167 abc	122,500 cdefg
Tersan 75 1,000 ppm	158,333 a	195,000 a	210,000 a
Tersan 75 2,000 ppm	155,000 a	190,833 ab	174,167 abc
Thane M-45 1,000 ppm	119,167 abcd	195,000 a	192,500 ab
Thane M-45 2,000 ppm	146,667 ab	190,000 ab	160,833 abcd
Cupravit 1,000 ppm	135,833 abc	168,333 abcd	138,333 bcdef
Cupravit 2,000 ppm	144,167 ab	172,500 abcd	148,333 bcde
Control 1	90,000 bcd	121,667 bcde	147,500 bcde
Control 2	85,000 cd	124,167 bcde	87,500 fg
Control 3	115,833 abcd	129,167 cdef	130,833 cdef
Control 4	104,167 abcd	155,833 a	112,500 abcd
CV (%)	25.42	23.00	20.24

Means in each column followed by the same letters are not significantly different (P = 0.05) according to DMRT.

(2) Nodule weight

There was no significant difference in fresh Rhizobium nodule weight of varieties SJ2, SJ4 and SJ5 during rainy or dry seasons from any chemical seed treatment (Table 3 and 4). However, in the dry season with SJ4, the control 2 treatments gave a significantly higher fresh nodule weight (2.51 g/10 plants) while there was no statistical difference among other treatments (Table 4).

Table 3 Average of fresh nodule weight of 3 soybean varieties after treatment with chemical seed treatments and *Rhizobium japonicum* during rainy season.

Chemicals	Varieties		
	SJ2	SJ4	SJ5
	Ave. of fresh nodules (g/10 plants)		
Aureomycin 500 ppm	0.70	0.65	0.65
Aureomycin 1,000 ppm	0.69	0.65	0.57
Dumocycline 500 ppm	0.66	0.61	0.62
Dumocycline 1,000 ppm	0.61	0.57	0.66
Terramycin 500 ppm	0.68	0.60	0.68
Terramycin 1,000 ppm	0.69	0.67	0.43
Agrimycin-100 500 ppm	0.64	0.93	0.78
Agrimycin-100 1,000 ppm	0.63	0.65	0.63
Tersan 75 1,000 ppm	0.85	0.74	0.72
Tersan 75 2,000 ppm	0.62	0.73	0.68
Thane M-45 1,000 ppm	0.70	0.70	0.72
Thane M-45 2,000 ppm	0.70	0.62	0.64
Cupravit 1,000 ppm	0.53	0.57	0.82
Cupravit 2,000 ppm	0.68	0.65	0.70
Control 1	0.76	0.69	0.78
Control 2	0.92	0.91	1.08
CV (%)	35.81	49.40	47.69

There is no statistical difference (P = 0.05) according to DMRT.

(3) 100-seed weight

There was no significant effect of chemical seed treatment on the weight of 100 soybean seeds of varieties SJ2, SJ4 and SJ5 during the rainy season (Table 5).

During the dry season, however, it was found for SJ2, that 2000 ppm Thane M-45 gave the highest 100-seed weight of 16.4 g which did not differ significantly from those of 1000 ppm Cupravit and 500 ppm Agrimycin-100 that gave the next highest weights of 15.6 and 15.5 g, respectively (Table 6). The application of 1000 ppm Aureomycin gave the lowest weight of 100 seeds of 13.2 g. Only the treatment with 2000 ppm Thane M-45 showed a significantly higher weight of 100 seeds than those of control-1 and -2.

In SJ4, 2000 ppm Thane M-45 and 500 ppm Agrimycin-100 gave the highest 100-seed weights of 17.2 and 17.0 g respectively. The lowest seed weight (13.7 g) was given by 500 ppm

Table 4 Average of fresh nodule weight of 3 soybean varieties after treatment with chemical seed treatments and *Rhizobium japonicum* during dry season.

Chemicals	Varieties		
	SJ2	SJ4	SJ5
	Ave. of fresh nodules (g/10 plants)		
Aureomycin 500 ppm	0.37	0.71 b	0.85
Aureomycin 1,000 ppm	0.27	0.80 b	0.43
Dumocycline 500 ppm	0.33	0.33 b	0.60
Dumocycline 1,000 ppm	0.33	0.49 b	0.61
Terramycin 500 ppm	0.22	0.57 b	0.24
Terramycin 1,000 ppm	0.27	0.46 b	0.94
Agrimycin-100 500 ppm	0.36	0.29 b	0.10
Agrimycin-100 1,000 ppm	0.29	0.71 b	0.29
Tersan 75 1,000 ppm	0.27	0.46 b	0.32
Tersan 75 2,000 ppm	0.37	0.41 b	0.31
Thane M-45 1,000 ppm	0.20	0.33 b	0.36
Thane M-45 2,000 ppm	0.27	0.41 b	0.61
Cupravit 1,000 ppm	0.42	0.40 b	0.63
Cupravit 2,000 ppm	0.22	0.51 b	0.47
Control 1	0.13	1.13 b	0.94
Control 2	0.06	2.51 a	1.31
CV (%)	66.97	68.72	84.11

Means in column of SJ4 followed by the same letters are not significantly different (P = 0.05) according to DMRT.

Dumocycline which was lower than those of control-1 and -2, which gave 100 seed weights of 14.8 and 14.3 g respectively (Table 6)

In SJ5, no statistical difference in any treatment was found (Table 6)

(4) Seed yield

The highest yield (1,725 kg/ha) of soybean variety SJ2 during the rainy season (Aug-Nov, 1984) was obtained by using 1000 ppm Thane M-45 which was not statistical different from 2000 ppm Thane M-45 and 2000 ppm Tersan 75 whose yields were 1,712 and 1,684 kg/ha respectively (Table 7). There were also no statistical differences between the results of these 3 treatments and control 1 & 2, which gave the yields of 1,470 and 1,437 kg/ha respectively. However, the yields of the above 3 treatments were significantly higher than 1000 ppm Dumocycline which gave the lowest yield of 1,121 kg/ha.

Table 5 Average of 100 seed weight of 3 soybean varieties grown in the field after treatment with chemical seed treatments and Rhizobium nodule bacteria during rainy season.

Chemicals	Varieties		
	SJ2	SJ4	SJ5
	Ave. of 100 seed weight (g)		
Aureomycin 500 ppm	13.9	15.0	15.3
Aureomycin 1,000 ppm	14.4	14.8	15.8
Dumocycline 500 ppm	14.2	14.8	15.4
Dumocycline 1,000 ppm	14.3	14.6	15.1
Terramycin 500 ppm	14.2	14.9	14.9
Terramycin 1,000 ppm	14.3	14.7	14.7
Agrimycin-100 500 ppm	14.1	14.8	15.6
Agrimycin-100 1,000 ppm	14.3	14.7	15.4
Tersan 75 1,000 ppm	14.3	15.0	15.4
Tersan 75 2,000 ppm	14.4	15.2	15.4
Thane M-45 1,000 ppm	14.6	14.8	14.3
Thane M-45 2,000 ppm	14.2	14.5	15.7
Cupravit 1,000 ppm	14.4	14.9	15.4
Cupravit 2,000 ppm	13.7	15.2	15.4
Control 1	13.6	14.4	15.5
Control 2	13.4	14.5	15.4
Control 3	13.6	13.1	13.9
Control 4	13.2	13.7	13.9
CV (%)	4.47	3.95	6.88

There is no statistical difference (P = 0.05) according to DMRT.

In SJ4, 2000 ppm Tersan 75 gave the highest yield of 1,858 kg/ha which did not differ statistically from 1000 ppm Tersan 75 (1,818 kg/ha). Both treatments also gave significantly higher yields than 1000 ppm Dumocycline which gave the lowest yield of 1,185 kg/ha. In addition, 1000 and 2000 ppm Tersan 75 gave significantly higher yield than control 1 & 2, whose yields were 1,438 and 1,365 kg/ha respectively.

In SJ5, 1000 ppm Tersan 75, 1000 ppm Agrimycin-100 and 2000 ppm Thane M-45 were found to give the highest yields of 1,716, 1,713 and 1,711 kg/ha respectively. The yields were higher than that of 1000 ppm Aureomycin which gave the lowest yield of 1,213 kg/ha. However, the results of the 3 treatments were not statistically

Table 6 Average of 100 seed weight of 3 soybean varieties grown in the field after treatment with chemical seed treatments and Rhizobium nodule bacteria during dry season.

Chemicals	Varieties		
	SJ2	SJ4	SJ5
	Ave. of 100 seed weight (g)		
Aureomycin 500 ppm	15.2 abc	14.7 bcde	14.9
Aureomycin 1,000 ppm	13.2 d	14.3 de	15.9
Dumocycline 500 ppm	14.1 bcd	13.7 e	16.6
Dumocycline 1,000 ppm	13.8 cd	15.6 abcd	16.1
Terramycin 500 ppm	14.8 abcd	14.6 cde	17.0
Terramycin 1,000 ppm	14.0 bcd	14.8 bcde	16.5
Agrimycin-100 500 ppm	15.5 ab	17.0 a	17.6
Agrimycin-100 1,000 ppm	14.3 bcd	16.5 abc	17.0
Tersan 75 1,000 ppm	15.2 abc	16.4 abcd	17.6
Tersan 75 2,000 ppm	15.3 abc	16.7 ab	17.7
Thane M-45 1,000 ppm	14.8 abcd	15.2 abcde	18.0
Thane M-45 2,000 ppm	16.3 a	17.2 a	16.6
Cupravit 1,000 ppm	15.6 ab	16.2 abcd	16.2
Cupravit 2,000 ppm	14.2 bcd	16.9 ab	15.3
Control 1	14.5 bcd	14.8 bcde	16.3
Control 2	14.6 bcd	14.3 cde	15.7
Control 3	13.7 e	13.9 cd	16.9
Control 4	14.0 bcd	13.9 cd	6.2
CV (%)	5.81	7.2	7.66

Means in each column followed by the same letters are not significantly different (P = 0.05) according to DMRT.

different from control-1, which gave a yield of 1,415 kg/ha, but they were still higher than Control 2 whose yield was 1,385 kg/ha (Table 7).

The result of soybean yield from the dry season experiment (Jan-Apr, 1985) revealed that for SJ2, 1000 ppm Tersan 75 gave the highest yield of 2,143 kg/ha which was not signigicantly different from 2,058 kg/ha of 1000 ppm Thane M-45, the next highest yield. However, both treatments gave higher yields than 1000 ppm Dumocycline, which gave the lowest yield of 708 kg/ha. As for 1000 ppm Tersan 75, the yield was higher than Control 1 & 2 which gave the yields of 1,378 and 1,234 kg/ha which 1000 ppm Thane M-45 did not differ from Control 1 & 2 (Table 8).

Table 7 Yield of 3 soybean varieties grown in the field after treating with chemical seed treatments and Rhizobium nodule bacteria during rainy season.

Chemicals	Varieties		
	SJ2	SJ4	SJ5
	Soybean yields (kg/ha)		
Aureomycin 500 ppm	1,376 bcde	1,727 abcd	1,261 cd
Aureomycin 1,000 ppm	1,235 de	1,396 defg	1,213 d
Dumocycline 500 ppm	1,522 abcd	1,259 fg	1,439 abcd
Dumocycline 1,000 ppm	1,127 e	1,185 g	1,249 cd
Terramycin 500 ppm	1,334 cde	1,576 abcdef	1,538 abc
Terramycin 1,000 ppm	1,334 cde	1,460 bcdefg	1,606 ab
Agrimycin-100 500 ppm	1,442 abcde	1,672 abcde	1,699 ab
Agrimycin-100 1,000 ppm	1,627 abc	1,553 abcdef	1,713 a
Tersan 75 1,000 ppm	1,625 abc	1,818 ab	1,716 a
Tersan 75 2,000 ppm	1,684 ab	1,858 a	1,611 ab
Thane M-45 1,000 ppm	1,725 a	1,796 abc	1,589 ab
Thane M-45 2,000 ppm	1,712 ab	1,744 abcd	1,711 a
Cupravit 1,000 ppm	1,547 abcd	1,652 abcde	1,659 ab
Cupravit 2,000 ppm	1,464 abcd	1,625 abcde	1,633 ab
Control 1	1,470 abcd	1,438 cdefg	1,415 abcd
Control 2	1,439 abcde	1,365 efg	1,385 bcd
Control 3	1,458 abcd	1,466 abcd	1,568 abcdef
Control 4	1,350 def	1,348 def	1,481 abcd
CV (%)	11.69	11.76	10.70

Means in each column followed by the same letters are not significantly different (P = 0.05) according to DMRT.

In SJ4, 2000 ppm Thane M-45, 500 ppm Agimycin-100, 1000 ppm Cupravit, 1000 ppm Tersan 75, 1000 ppm Agrimycin-100 and 2000 ppm Cupravit gave the highest yield of 2,201 ; 2,172 ; 2,119 ; 2,089 ; 2,075 and 2,058 kg/ha respectively. All treatments gave significantly higher yields than 1000 ppm Dumocycline, which gave the lowest yield of 1,229 kg/ha and also control 1 & 2, whose yields were 1,385 and 1,436 kg/ha, respectively (Table 8).

In SJ5 1000 and 2000 ppm Tersan 75 gave the highest yields of 2,021 and 1,987 kg/ha, respectively. These treatments gave significantly higher yields than 1000 ppm Aureomycin, which gave the lowest yield of 1,035 kg/ha, and control 1 (1,473 kg/ha) and control 2 (1,412 kg/ha) (Table 8).

Table 8 Yield of 3 soybean varieties grown in the field after treatment with chemical seed treatments and Rhizobium nodule bacteria during dry season.

Chemicals	Varieties		
	SJ2	SJ4	SJ5
	Soybean yields (kg/ha)		
Aureomycin 500 ppm	1,806 abcd	1,794 abc	1,468 bcd
Aureomycin 1,000 ppm	1,008 ef	1,659 abc	1,035 d
Dumocycline 500 ppm	1,271 cdef	1,343 bc	1,695 ab
Dumocycline 1,000 ppm	708 f	1,229 c	1,059 cd
Terramycin 500 ppm	1,671 abcde	1,779 abc	1,809 ab
Terramycin 1,000 ppm	1,129 def	1,348 bc	1,574 ab
Agrimycin-100 500 ppm	1,668 abcde	2,172 a	1,735 ab
Agrimycin-100 1,000 ppm	1,616 abcde	2,075 a	1,815 ab
Tersan 75 1,000 ppm	2,143 a	2,089 a	2,021 a
Tersan 75 2,000 ppm	1,989 abc	1,674 abc	1,987 a
Thane M-45 1,000 ppm	2,058 ab	1,835 ab	1,752 ab
Thane M-45 2,000 ppm	1,686 abcd	2,201 a	1,781 ab
Cupravit 1,000 ppm	1,917 abc	2,119 a	1,771 ab
Cupravit 2,000 ppm	1,944 abc	2,058 a	1,504 bc
Control 1	1,378 bcdef	1,385 bc	1,473 bcd
Control 2	1,324 bcdef	1,436 bc	1,412 bcd
Control 3	1,370 bcdef	1,362 bcdef	1,337 bc
Control 4	1,369 bcdef	1,428 bc	1,392 bcd
CV (%)	24.48	17.07	15.22

Means in each column followed by the same letters are not significantly different (P = 0.05) according to DMRT.

DISCUSSION

The result of this study on the effect of 7 chemical seed treatments, including adjuvant, on the Rhizobium nodule bacteria under laboratory conditions revealed that all chemicals except Agrimycin applied to strain TH7 at 250-1500 ppm inhibited the growth of Rhizobium. It was apparent from these results that the lower concentration of oxytetracycline (1.5%) in Agrimycin did not inhibit Rhizobium strain TH7, while at the higher concentration in Terramycin there was complete inhibition. It was also indicated that 18.8% streptomycin in Agrimycin was unable to inhibit Rhizobium strain TH7 as well. As for control-1 using adjuvant (Shellestral), it contained the mixture of detergent which caused the substance to stick and spread over the seed coat reducing water surface tension and such detergent would inhibit the growth of every strain of Rhizobium nodule bacteria.

Under field conditions, it was found that all 3 soybean varieties treated with fungicides had higher average % seed germination than control treatments and seed treated with antibiotics. These results were similar to those reported by Allington *et al* (1945).

The investigation of 100-seed weight revealed no difference among treatments in the wet season since there was only a small significant difference in the percentage of infected leaves (Prathungwong and Choochoa, 1988), thus causing no evident difference in 100-seed weight. However, according to Prathuangwong and Choakhane (1984), where leaf area was infected more than 50% and caused defoliation, it would be expected to cause distinct differences between the 100-seed weight of infected and healthy plants.

The average weight of fresh Rhizobium nodule in all treatments, including the control, in both the rainy and dry seasons was very low. The preparation of the field plots at the National Maize and Sorghum Research Center (Suwan Farm) included the application of 16-20-0 compound fertilizer at the rate of 75 kg/rai. As a result of this, it would be expected that these would be a great quantity of nitrogen in soil which in turn would cause less Rhizobium activity. The results were similar to that reported by Thronton (1935) who suggested that if there was nitrate fertilizer in the root vicinity, there was a reduction in the number of nodules. Richardson *et al* (1957) also reported that if nitrogen in the form of No_3^- and NH_4^+ was higher than 60 ppm, the amount of nodules would decrease and Bhangoo and Albritton (1976) found that if nitrogen fertilizer was applied at more than 224 kg N/ha, the nitrogen fixing process in soybean nodule bacteria would be totally inhibited. However, Rhizobium strains resident in the field may be one of the important factor to cause no statistical difference in fresh nodule weight. In the rainy season, the weight of fresh Rhizobium nodules were higher than those in the dry season. This may have been due to soybean, as well as other beans, being grown continuously for several seasons in the same field in the case of the rainy season experiment whereas the dry season experiment was conducted in the corn fields, where the amount of Rhizobium would be expected to be lower than in the area used in the rainy season. However, the weight of fresh nodules of the untreated control treatment tended to be higher than those of the other treatments, even though no statistical difference occurred, except in SJ4 in the dry season where control-2 differred significantly from the other treatments, with no difference among treatments. This suggested that every kind and level of concentration of the seed treatment chemical would equally inhibit the growth of Rhizobium.

The investigation on soybean yield revealed that the use of fungicides gave higher yield than the antibiotics and the controls, even though there was no difference in the percentage of infected leaves between the control and chemical treatments (Prathuangwong and Choochoa, 1988). It was found that the only factor affected yield was the number of plants. When soybean seeds were treated with fungicides, the percentage of seed germination and the number of plants established was higher than those of the antibiotics and the controls. These results were similar to those reported by Ellis *et al* (1975) and Prathuangwong and Choakhane (1984) which suggested that some antibiotics had toxic effects on both seed germination and leaves of soybean. It was also observed that inoculation with Rhizobium gave higher average yield for every variety in every treatment than without Rhizobium, both in the rainy and dry seasons. It was possible that the quantity of nitrogen fertilizer applied to the soil by the National Maize and Sorghum Research Center was not high enough, thus Rhizobium inoculation caused an increase in nitrogen fixation of soybean which in turn gave healthy and higher yield than without Rhizobium. In addition, it also showed that although the chemicals for seed treatment were generally capable of inhibiting Rhizobium growth in the laboratory, there were some strains of some mutant colonies which tolerated chemicals.

However, the effect of chemical seed treatment on yield was mainly a result of differences in the number of plants established per unit area. Therefore, the beneficial effect on chemical seed treatment on yield may be due to the effect on the better control of other pathogen residents in the plots which were affecting seedling establishment than of bacterial pustule where the less percentage of infected seed was found (Prathuangwong and Choochoa, 1988).

CONCLUSION

Seven chemicals namely Aureomycin, Dumocycline, Agrimycin-100, Terramycin, Tersan 75, Thane M-45 and cupravit at the concentration of 250 ppm were found to inhibit the growth of every strain of Rhizobium, except for Agrimycin-100 which did not affect TH7 strain, unless the concentration was increased to 2000 and 2500 ppm. Under field conditions, there was no difference in the weight of fresh nodule between chemical treatments and controls. While the 100-seed weight was not affected by Rhizobium inoculation or chemicals, the total seed yield in both wet and dry season was significantly increased by chemicals. This was because certain fungicides applied to seeds before planting caused an increase in the percent germination.

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